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Using Polystyrene Beads to Estimate Feeding Selectivity and Potential Energetic Consequences in the Rotifer, *Brachionus plicatilis*

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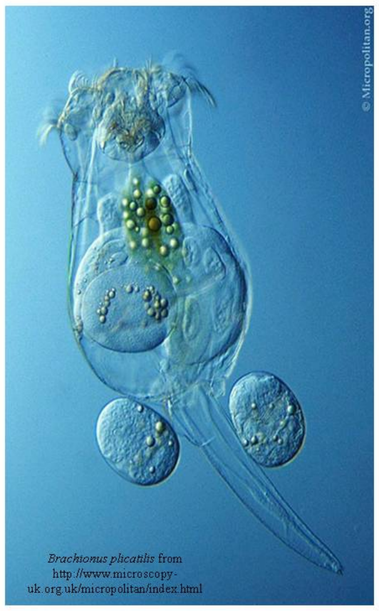
Abstract

Rotifers are free-swimming aquatic animals that feed by collecting particles through the use of cilia that are arranged in two circular whorls. Specimens of *Brachionus plicatilis* exposed to 6 µm and 0.5 µm polystyrene beads (together or separately) captured the particles at significantly different rates. Calculation of the Jacob's Selectivity Index (D) revealed a significant selectivity for the larger (6 µm) beads, over the smaller (0.5 µm) beads ($p < 0.001$), and the presence of 6 µm beads negatively affected the capture of 0.5 µm beads ($p<0.001$). We propose that the clearance rate (volume cleared of particles / time) of 0.5 µm beads approximates fluid transport rates through the digestive system, and represents the mechanism of capture of bacteria-sized particles. This rate of 0.5 µm bead clearance appears to be efficient enough to meet metabolic demand through the uptake of dissolved organic matter at the concentration of 0.01 µg/µl, but still too slow to be energetically beneficial in obtaining enough bacteria ($<1x10^8$/ml).

Introduction

The Phylum Rotifera is a diverse group of multicellular, microscopic animals that are common inhabitants of freshwater, estuarine, and marine habitats. Locomotion and feeding of rotifers are accomplished through the activity of one or two anterior ciliated bands (the corona) that direct water containing particulate food toward their oral field. For species that use the conora to concentrate particulate foods (i.e., *Philodina* and *Brachionus*) the spectrum of particles ranges from 0.3 µm to 12 µm (Vadstein et al., 1993; Rothhaupt et al., 1990). However, in a mixture of different sized particles, not all sizes are collected at the same frequency. Baer et al. (2008) reported that clearance rate of 2 µm particles by *Brachionus plicatilis* was consistently low (< 0.5 µL/h), and independent of particle concentration and also that this species preferentially captured 4.5 µm beads. Starkweather et al. (1979) earlier reported that the related species, *B. calyciflorus* could not efficiently collect small beads (0.5-1.0 µm).

Through new comparisons of clearance rates of the 6 µm and 0.5 µm beads, we extended studies relating the effect of particle size to feeding rates of *B. plicatilis*. We then used the measured clearance rates to compare the predicted energetic contribution of small particles (bacteria) and dissolved organic matter to the metabolism of an adult *B. plicatilis*.



Materials/ Methods

Resting Rotifers™ were obtained from Florida Aqua Farms Inc. and cultures were created in beakers of filtered saltwater covered and placed under continuous light at room temperature. The rotifers were fed daily with Roti-Rich™.

Rotifers were transferred to filtered seawater for 12 to 18 hours to clear their digestive tract. Prior to each experiment polystyrene beads (6 µm and 0.5 µm) were mixed with an equal amount of a 50 mg/mL solution of bovine serum albumin in order to coat the beads and reduce bead clumping. This mixture was centrifuged, decanted, resuspended in filtered water, and then sonicated to separate any aggregated beads. Rotifers were incubated for 10 minutes in 10 mL of filtered seawater (≤ 5 rotifers/mL) with beads at initial concentrations of 44210 beads/mL (6 µm) or 763614 beads/mL (0.5 µm). The rotifers were collected on a small mesh sieve, relaxed in carbonated water, and then fixed using 2.5% of paraformaldehyde. A Nikon E600 compound microscope equipped for fluorescence microscopy was used to count the number of particles ingested. Clearance rates (volume of water 'cleared' of particles) were calculated by multiplying the number of ingested beads by the bead concentration and dividing by time.

Two separate experiments were conducted to quantify a difference in *B. plicatilis'* preference for the two bead sizes. We used Jacob's Selectivity Index (D) (from Baer et al., 2008) to estimate the degree of particle size selection.

Results

- All experiments conducted with both bead sizes present show a significantly higher 6 µm clearance rate (3.91±2.01 µL/h) than 0.5 µm bead clearance rate (0.27±0.25 µL/h; Figure 1)
- In 87% of experiments, the clearance rate of the 0.5 µm beads is significantly lower in the presence (0.26 ± 27 µL/h, n=82) than in the absence (0.53 ± 0.27 µL/h, n=21) of 6 µm beads ($p<0.001$; Figure 3).
- The clearance rate of the 6 µm beads is significantly lower when 0.5 µm beads are present (3.63±4.45 µL/h, n=136) compared to when only 6 µm beads are available (4.91±6.01 µL/h, n=247; $p=0.029$).
- There is a significant correlation between 6 µm beads and 0.5 µm beads (Figure 2; $p<0.001$).
- The Jacob's Index for 6 µm beads (0.65 ± 0.49) was significantly greater than 0 ($p<0.001$), and indicates a strong selective preference for 6 µm beads.

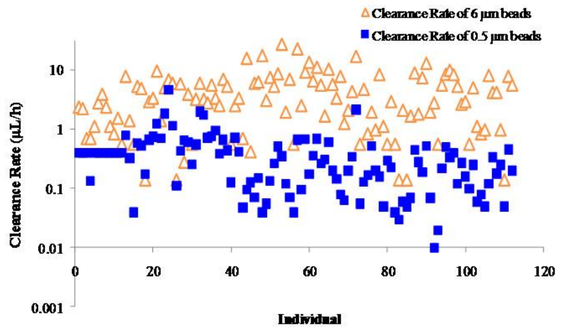


Figure 1: **Simultaneous Clearance Rates of 6 µm and 0.5 µm Beads by *Brachionus plicatilis*.** In all experiments (n=112) the clearance rate of 6 µm beads was significantly higher than the clearance rate of 0.5 µm beads.

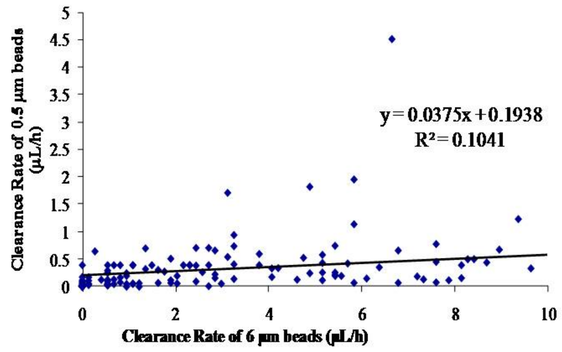


Figure 2: The Relationship between clearance rates of 6 µm and 0.5 µm beads (µL/h).

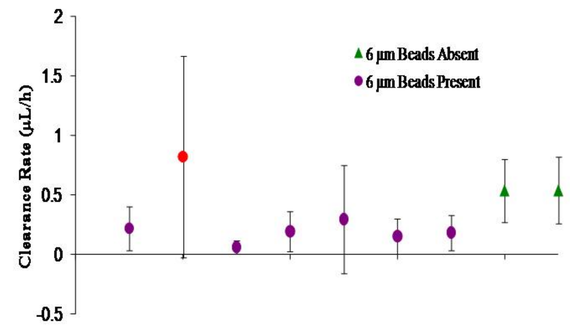


Figure 3: The Clearance Rates of 0.5 µm Beads in the Presence (Circle Symbols) and Absence (Triangle Symbols) of 6 µm Beads. The values are the means of different experiments. All circles of the same color (excluding red circle experiment) have clearance rates of 0.5 µm beads that are significantly higher when 6 µm beads are absent ($p<0.001$).

Nutrient Source	Concentration (mg or cells/mL)	0.5 µm Bead Clearance Rate (µL/h)	Respiration Rate (µJ/h)	% Compensation at 100% efficiency	% Compensation at 50% efficiency
DOM	0.1	0.533	49.781	1873.71%	936.85%
DOM	0.01	0.533	49.781	187.37%	93.69%
DOM	0.001	0.533	49.781	18.74%	9.37%
Bacteria	$1x10^6$	0.533	49.781	0.4156%	0.2078%
Bacteria	$1x10^7$	0.533	49.781	4.1561%	2.0780%
Bacteria	$1x10^8$	0.533	49.781	41.5610%	20.7805%

Table 1: Potential Energetic Contribution of Dissolved Organic Matter (DOM) and Bacteria to *Brachionus plicatilis*. Respiration rate was measured at 20°C (Doohan 1973).

Discussion

The notable differences in clearance rates of the different sized beads by *Brachionus plicatilis* has lead to some alternate interpretations of the potential importance of bacteria and dissolved organic matter (DOM) to the metabolism of this rotifer. The differences in the measured clearance rates of the large and small particles suggest that the rotifer's feeding apparatus handles and ingests these particles in dissimilar ways. Since the smaller beads do not appear to be actively collected at such a high rate, we propose that they represent the rate of fluid flow through the rotifer digestive system.

Using a clearance rate equal to the average 0.5 µm bead clearance rate, we estimated that benefits of DOM uptake can be sufficient to supply the rotifer metabolic demands at DOM concentrations ≥ 0.01 mg/mL. In contrast, the consumption of bacteria at ambient concentrations (ca. $1x10^6$ cells/mL) at this same rate could supply only 0.42% of their metabolic rate (Table 1). The presence of the 6 µm beads was found to greatly affect the clearance rates of 0.5 µm beads, indicating that passive fluid flow is greater when larger particles (6 µm beads) are not present. Future research on additional rotifer species should be completed to further test the "fluid flow" hypothesis and its influence on the ability of rotifers to obtain nutrients in the form of DOM and bacteria.

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